



S3 Fig. Dnmt1 forms dimers in solution via interaction with the TS domain of Dnmt1.

A) Recombinant Dnmt1 was loaded on a superdex200 gel filtration column (GE Healthcare) in running buffer (200mM NaCl/2M NaCl, 20mM Tris, pH 8.0; 1mM MgCl₂, 10% glycerol). Upper panel. Dnmt1 in running buffer supplemented with 200mM NaCl. Lower panel. Dnmt1 in running buffer with 2M NaCl.

B) Dnmt1 (30μg) was adjusted to the indicated SDS concentration and either incubated for 30min at 37°C or directly loaded on a superose6 gel filtration column (GE Healthcare) in running buffer (150mM NaCl, 20mM Tris, pH 7.5; 1mM EDTA, 10% glycerol) supplemented with the indicated concentrations of SDS. For both columns, 500μl fractions were collected, TCA precipitated and subjected to SDS-PAGE and Western Blot analysis (detection with the Dnmt1 specific antibody 2G3). Molecular weight standards and fractions are indicated.

C) Dnmt1 co-immunoprecipitates the TS domain. Recombinant Dnmt1 was expressed in insect cells, purified first by Ni-NTA affinity chromatography and second by chromatography on ResourceQ material (GE Healthcare) (lanes 1 and 2). Purified MBP-TS was cleaved with TEV Protease (lane 4) and incubated with the Dnmt1 specific antibody 2E8 coupled to proteinG beads. 2% of the flowthrough and 50% of the beads were loaded on a 6% SDS-PAGE (lanes 5 and 6). Recombinant Dnmt1 (lane 8) was incubated with a 50 fold excess of the TS domain for 1h at 30°C. The reaction was incubated with the 2E8 antibody coupled to proteinG beads and 10% of the flowthrough and 50% of the beads were loaded on a 6% SDS-PAGE (lane 9 and 10). Proteins were stained with Coomassie Blue. The positions of Dnmt1, the antibody heavy chain (HC), the Maltose Binding Protein (MBP) and the human TS domain are indicated.